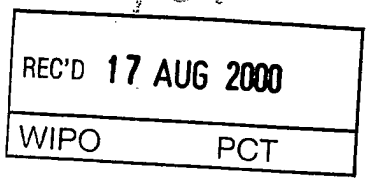
09 JUNE 2000

INVESTOR IN PEOPLE

GB00/2406



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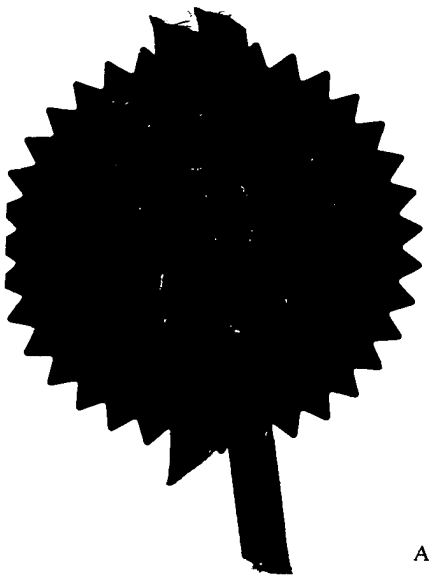

Signed Andrew Gerry

Dated 18 JUL 2000

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Patents Form 1/77

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11 JUN 1999

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11 JUN 1999

The Patent Office

Cardiff Road
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1. Your Reference

P.6195.GBA

2. Patent application number
(The Patent Office will fill in this part)

9913487.6

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Dr Ramin PIRZAD
40 Nursery Gardens
St. Ives
CAMBS PE17 6NL

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation

7678238001

4. Title of the invention

DUST MITE DETECTION

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

MAGUIRE BOSS
5 Crown Street
St. Ives
Cambridgeshire
PE17 4EB

Patents ADP number *(if you know it)*

07188725001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day/month/year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day/month/year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body:)
See note (d)

NO

Patents Form 1/77

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Continuation sheets of this form	
Description	8
Claims(s)	
Abstract	
Drawing(s)	2

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature Maguire Boss Date 10/06/99

MAGUIRE BOSS

12. Name and daytime telephone number of person to contact in the United Kingdom PAUL J EVENS Tel: 01480 301588

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DUST MITE DETECTION

The present invention relates to dust mite detection, and more particularly to a method and apparatus for indicating dust mite activity in dust samples.

It is estimated that up to 80% of the dust particles illuminated by incident sunlight and made visible to the naked eye in a domestic environment are derived from skin. In a warm environment, dust mites feed on skin-derived dust particles, breaking it down by using proteases in their digestive system. Such proteases are found in not insignificant levels in dust mite faeces, and it is now established that it is excreted proteases which act as allergens to individuals who are liable to have an allergic response to house dust. Concentrations of excreted protease are found in relatively high levels in carpets, bedding, pillows and mattresses, all of which provide a suitable environment for dust mites to thrive.

It is known to test house dust in order to determine quantitatively levels of the house dust mite allergen. According to one patent, US 4806490, a dust sample is suspended in an aqueous-alcoholic alkali metal hydroxide

solution to dissolve or leach out aromatic compounds such as guanine excreted by dust mites, and the resulting solution is mixed with an aromatic diazo compound. A reaction between the aromatic diazo compound and any excreted aromatic compounds in the solution produces a colour change, with the intensity of the new colour being indicative of the level of excreted proteases in the house dust.

According to a first aspect of the present invention, there is provided a method of determining dust mite activity in dust, comprising: providing a dust sample; extracting from the dust sample at least one component selected from the group consisting of proteases, amines, amino acids and peptides; reacting the extracted at least one component with 2, 4, 6-trinitrobenzene sulphonic acid (hereinafter referred to as TNBSA) in a solution; determining the colour of the solution; and providing an indication of dust mite activity in dependence upon the colour determined.

The present applicant has appreciated that in addition to proteases, dust mites excrete the by-products of skin breakdown, including amine compounds, amino acids and relatively small chain peptides, e.g., glycylglycine. In part, the present invention is directed to detecting some of the more abundant, and in some cases chemically less complex, by-products to give an indication of the allergen concentration, rather than targeting one specific compound (e.g., guanine) or type of compounds

(e.g., aromatic compounds). This will enable individuals to test particular environments, e.g., individual rooms in a domestic situation to establish that environment's propensity for inducing an allergic response.

The at least one component may be extracted by bringing the dust sample into contact with a surface active agent (surfactant). Any dust sample solid residues may be separated from the surfactant prior to reacting with TNBSA. The surfactant may be an aqueous solution comprising sodium dodelcy sulphate, possibly present in an amount of about 5 wt%. The aqueous solution may be alkaline and may also comprise sodium hydrogen carbonate. The dust sample solid residues may be separated by filtration. Removing the solid residues facilitates accurate colour determination by reducing the amount of opaque material in the solution.

The colour may be determined by comparison with at least one reference colour. The comparison may be with a plurality of different colour references, each selected from the spectrum of colours or range of colour hues attainable. The different colour references may be selected to indicate at least three different kinds of dust mite activity, perhaps corresponding to a macroscopic gradation such as low, medium and high activity.

The reaction mixture may be preserved by using a stopping agent, e.g., hydrochloric acid, after a pre-selected incubation or dwell time, e.g., about 2 minutes.

The method may further comprise exposing the dust sample or the extracted at least one component to a protease substrate. Exposure to the protease substrate, which may include a protein, for a given period may enable proteases in or from the dust sample to be broken down. The resulting amino acids and peptides may then be tested by reacting with TNBSA from which the colour determination would be a direct indication of allergen levels in the dust sample. The given period may be 15 minutes.

In order to give reproducable results, the dust sample may be of a predetermined size, e.g., by weight or by volume. The dust sample may be collected by a suction device, perhaps over a predetermined area or time. Variations in the dust sample size may be tolerated since the method represents a gross contamination test, so exact measurements of the dust samples are not necessarily essential.

In accordance with a second aspect of the present invention, there is provided a method of determining dust mite activity in dust, comprising: providing a dust sample; extracting from the dust sample at least one component selected from the group consisting of aliphatic amines and aliphatic amino acids; determining the relative concentration of the extracted at least one component; and providing an indication of dust mite activity in dependence upon the relative concentration determined.

The relative concentration may be determined by employing a colour indicator sensitive to aliphatic amines and amino acids. The colour indicator may comprise TNBSA.

Any by-products of skin breakdown, particularly aliphatic amines and aliphatic amino acids, present in the dust sample may be linked to dust mite activity. The higher the levels of the by-products in the dust sample, the higher the dust mite activity may be assumed to be. High levels of dust mite activity will produce a correspondingly high amount of protease - the allergens which are responsible for providing the allergic reaction to house dust in certain individuals.

In accordance with a third aspect of the present invention, there is provided apparatus for use in a domestic environment for determining dust mite activity. The apparatus may comprise a kit comprising a first chamber comprising a surfactant for extracting from a dust sample at least one component selected from the group consisting of proteases, amines, amino acids and peptides; a second chamber comprising TNBSA; means for determining the colour of a solution resulting from reacting the extract-containing surfactant and the TNBSA; and means for indicating relative level of dust mite activity in the dust sample based on the colour determination.

The apparatus may further comprise a filter for filtering dust sample solid residues from the surfactant

before reacting with the TNBSA. One of the two chambers may have the capacity to receive the contents of the other chamber. Preferably, the second chamber has the capacity to hold the TNBSA and the surfactant.

The colour determining means may comprise at least one colour reference, against which the colour of the solution may be compared. The indicating means may comprise a scale, e.g., low, medium and high activity, which is linked to the colour evaluated. For example, if the colour of the solution is determined by eye as being about the same as the colour reference, this could correspond to medium dust mite activity. Divergence either side of the colour reference would then correspond to low or high activity as appropriate.

The apparatus may further comprise a third chamber comprising a stopping reagent to limit the reaction between the extract-containing surfactant and TNBSA.

An embodiment of the invention will now be described with reference to the accompanying drawings, in which:

Figure 1 shows schematically apparatus for determining dust mite activity in accordance with the present invention; and

Figure 2 shows schematically the use of apparatus shown in figure 1.

The apparatus 10 of figure 1 comprises three parts: an upper part 12 which contains in a first chamber 14 0.10 litres of a 0.1M solution of sodium hydrogen carbonate containing 5 wt% of sodium dodelcy sulphate; a

middle part 16 which is a snug but sliding fit in both the upper part 12 and the remaining part; and a lower part 18 which contains a tablet of TNBSA and a stopping reagent of 1.0M hydrochloric acid. The solution in the first chamber 14 is sealed in the upper part 12 by a frangible seal 20. The middle part 16 comprises a filter 22 above which is provided a cup 24 for receiving a dust sample. The middle part 16 has a leading profile 26 which is pointed to facilitate breaking the frangible seal 20. A second chamber 27 is formed by the middle and lower parts. The lower part 18 includes a frangible seal 28 disposed between the tablet of TNBSA and the stopping reagent which is sealed in a third chamber 29.

The use of the apparatus 10 is now described in stages with reference to figure 2:

Stage 1 A sample of dust of predetermined size is placed in cup 24.

Stage 2 The middle part 16 is inserted into the upper part 12, such that the profile 26 ruptures the seal 20.

Stage 3 The solution in the first chamber comes into contact with the dust sample. Any chemicals including amines, amino acids and peptides present in the dust sample are extracted and pass through filter 22 and into the second chamber where they come into contact with the tablet of TNBSA.

Stage 4 After about 2 minutes, the middle part 16 is pushed far enough into the lower part 18 to rupture

seal 28, enabling the stopping reagent in the third chamber 29 to prevent further reaction. The colour of the resulting solution is compared with a colour key which is calibrated to give an indication of the level (e.g., low, medium or high) of dust mite activity in the dust sample.

Example

A dust sample was collected from an old mattress (where dust mite activity may be expected to be high), and a blank sample and test samples of GlycylGlycine in varying concentrations (20-200 micro-grams) were used as controls. The dust, blank and test samples were washed with 0.1M $NaHCO_3$ 0.5M NaCl (pH 8.3)and then tested with TNBSA of various concentrations e.g., diluted to 1 part in 10, 1 part in 50 and 1 part in 100. It was found that a dilution of 1 part in 50 was the optimum dilution for sensitivity and blank colour. Using such a dilution, the experiment yielded visual results for both the dust and all test samples, but not the blank sample. The visual results could then be assessed and compared to give an indication of dust mite activity in the old mattress.

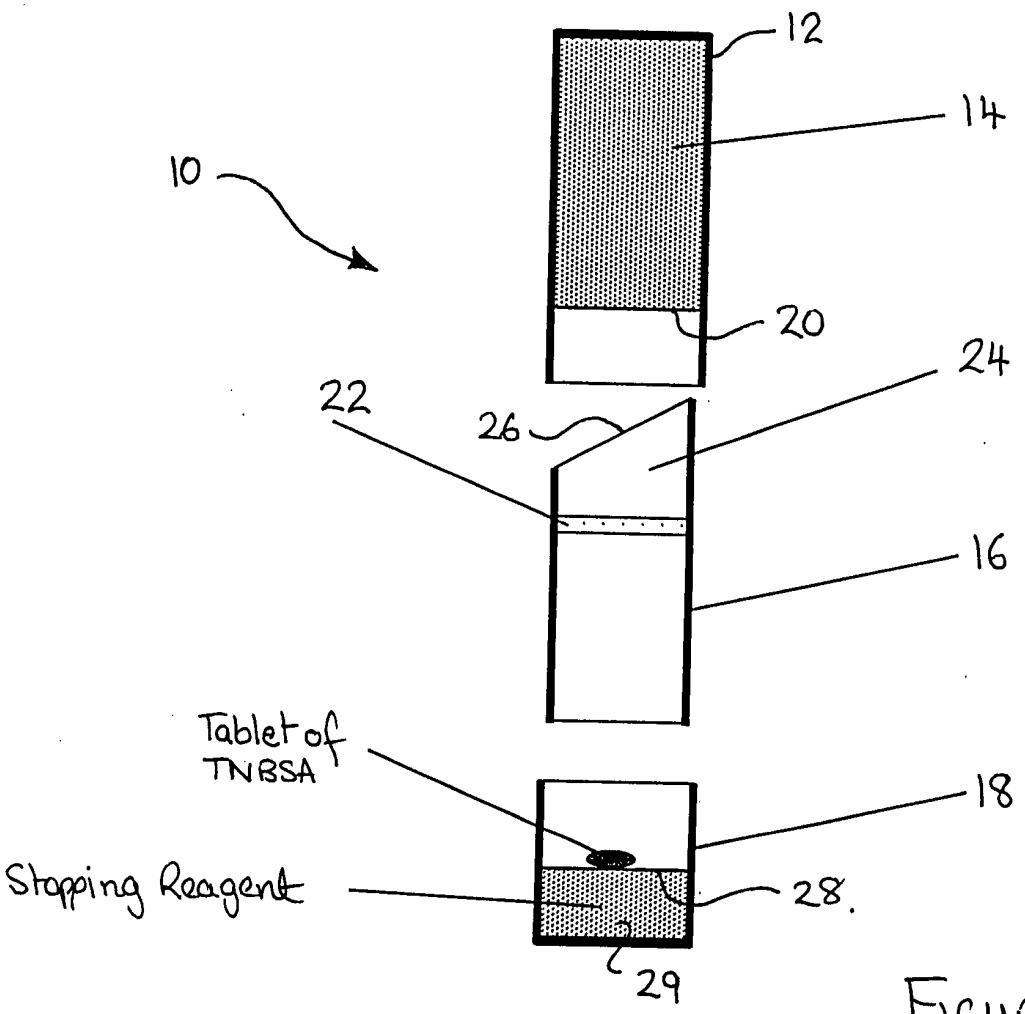
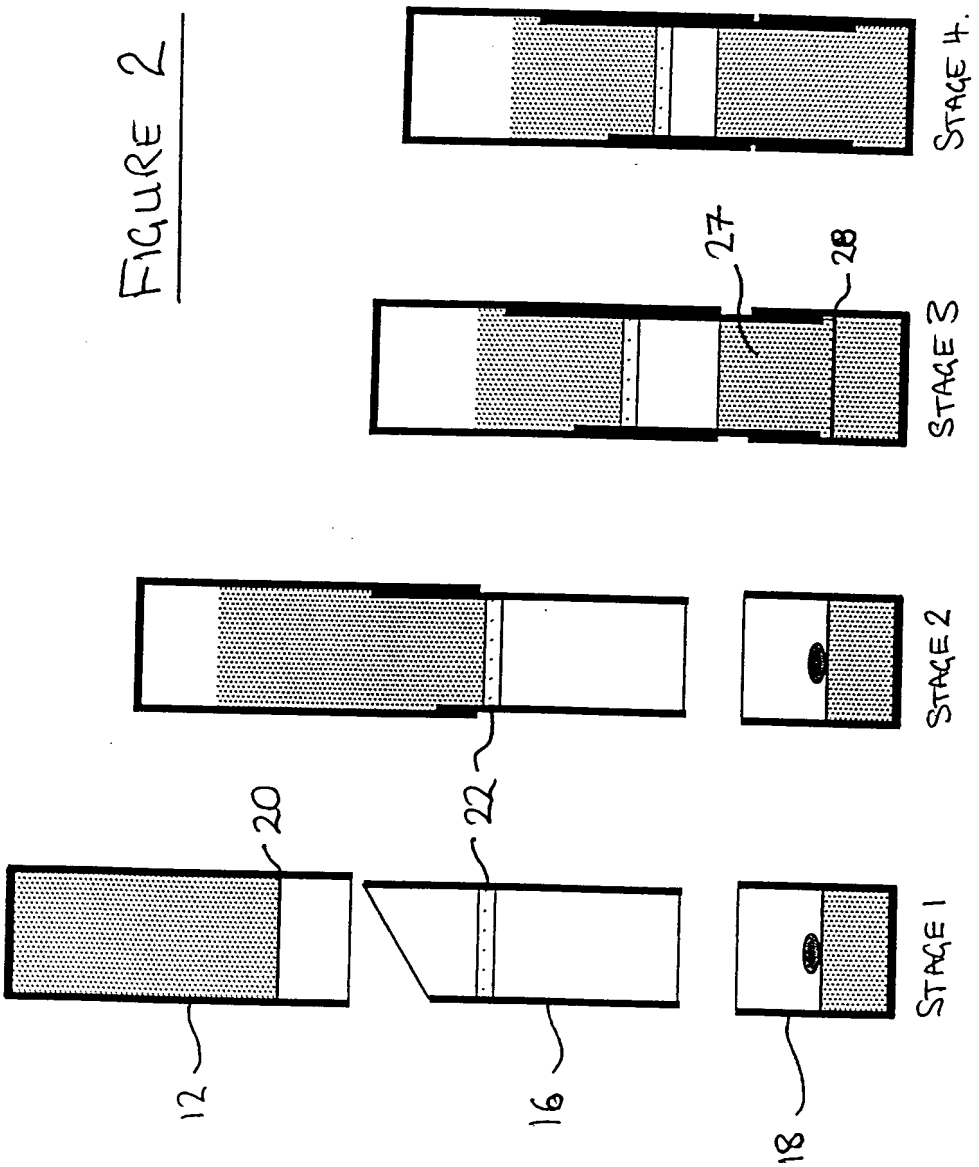

12
14
10
20
24
Sample cup holding
approximately 1g of dust
22
26
16
Tablet of
TNBSA
18
Stopping Reagent
28.
29

FIGURE 1

FIGURE 2



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